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Enclosed please find a provisional application for United States patent as identified
below:

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Title: METHODS FOR TREATING AMYLOID DISEASES

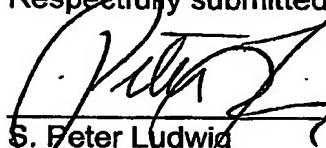
including the items indicated:

1. Specification and 20 claims: 2 indep.; 18 dep.; multiple dep.
2. Drawings, 1 sheets (Fig. 1)
3. Assignment for recording to:
4. Applicant claims small entity status
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PROVISIONAL PATENT APPLICATION COVER SHEET

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METHODS FOR TREATING AMYLOID DISEASES

1. FIELD OF THE INVENTION

The present invention relates to methods for treating human amyloid disease. Specifically, this invention relates to methods of reducing the blood levels of A β peptides by, e.g., the administration of compounds associated with A β , or the dialyzation of blood through a column or membrane to remove free A β .

2. BACKGROUND OF THE INVENTION

Amyloid disease, or amyloidosis, is characterized by the accumulation of a peptide, specifically the amyloid-beta (A β) peptide, existing as abnormal insoluble cross- β sheet fibrils or amyloid deposits in the affected organs. Amyloid diseases include, but are not limited to, Alzheimer's disease, type 2 diabetes, Huntington's disease, Parkinson's disease, chronic inflammation and cystic fibrosis. Amyloidosis is also a common and serious complication of long-term hemodialysis for end-stage renal failure. Amyloidosis - in which amyloid deposits are the direct cause of death - is responsible for about one per thousand of all deaths in developed countries.

Alzheimer's Disease (AD) is the most common form of late-life dementia in adults (Ghiso *et al.*, 2002), constituting the fourth leading cause of death in the United States.

Approximately 10% of the population over 65 years old is affected by this progressive degenerative disorder that is characterized by memory loss, confusion and a variety of cognitive disabilities.

Neuropathologically, AD is characterized by four major lesions: a) intraneuronal, 5 cytoplasmic deposits of neurofibrillary tangles (NFT), b) parenchymal amyloid deposits called neuritic plaques, c) cerebrovascular amyloidosis, and d) synaptic and neuronal loss. One of the key events in AD is the deposition of amyloid as insoluble fibrous masses (amyloidogenesis) resulting in extracellular neuritic plaques and deposits around the walls of cerebral blood vessels. The major constituent of the neuritic plaques and congophilic angiopathy is amyloid β ($A\beta$), 10 although these deposits also contain other proteins such as glycosaminoglycans and apolipoproteins.

Evidence that amyloid may play an important role in the early pathogenesis of AD comes primarily from studies of individuals affected by the familial form of AD (FAD) or by Down's syndrome. Down's syndrome patients have three copies of the APP gene and develop 15 AD neuropathology at an early age (Wisniewski et al., 1985). Genetic analysis of families with hereditary AD revealed mutations in chromosome 21, near or within the $A\beta$ sequence (Ghiso et al., 2002), in addition to mutations within the presenilin 1 and 2 genes. Moreover, it was reported that transgenic mice expressing high levels of human mutant APP progressively develop amyloidosis in their brains (Games et al., 1995). These findings appear to implicate 20 amyloidogenesis in the pathophysiology of AD. In addition, $A\beta$ fibrils are toxic to neurons in culture (Yankner et al., 1989) and to some extent when injected into animal brains (Sigurdsson et al., 1996 and 1997).

Furthermore, several other pieces of evidence suggest that the deposition of $A\beta$ is 25 a central triggering event in the pathogenesis of AD, which leads subsequently to NFT formation and neuronal loss. The amyloid deposits in AD share a number of properties with all the other cerebral amyloidoses, such as the prion related amyloidoses, as well as the systemic amyloidoses. These characteristics are: 1) being relatively insoluble; 2) having a high degree of β -sheet secondary structure, which is associated with a tendency to aggregate or polymerize; 3) ultrastructurally, the deposits are mainly fibrillary; 4) the presence of certain amyloid-associating

proteins such as amyloid P component, proteoglycans and apolipoproteins; and 5) deposits show a characteristic apple-green birefringence when viewed under polarized light after Congo red staining.

The same peptide that forms amyloid deposits in the AD brain was also found in a soluble form ($sA\beta$) normally circulating in human body fluids (Seubert et al., 1992 and Shoji et al., 1992). $sA\beta$ was reported to pass freely from the brain to the blood (Ji et al., 2001; Shibata et al., 2000; Ghersi-Egea et al. 1996). Zlokovic et al. (1994), reported that the blood-brain barrier (BBB) has the capability to control cerebrovascular sequestration and transport of circulating $sA\beta$, and that the transport of $sA\beta$ across the BBB was significantly increased in guinea pigs when $sA\beta$ was perfused as a complex with apolipoprotein J (apoJ). The $sA\beta$ -apoJ complex was found in normal cerebrospinal fluid (CSF; Ghiso et al., 1994) and *in vivo* studies indicated that $sA\beta$ is transported with apoJ as a component of the high density lipoproteins (HDL) in normal human plasma (Koudinov et al., 1994). It was also reported by Zlokovic et al. (1996), that the transport of $sA\beta$ from the circulation into the brain was almost abolished when the apoJ receptor, gp330, was blocked. It has been suggested that the amyloid formation is a nucleation-dependent phenomena in which the initial insoluble "seed" allows the selective deposition of amyloid (Jarrett et al., 1993).

Therapeutic strategies proposed for treating Alzheimer's disease and other amyloid diseases include the use of compounds that affect processing of the amyloid- β precursor protein (Dovey et al., 2001), or that interfere with fibril formation or promote fibril disassembly (Soto et al., 1998; Sigurdsson et al., 2000; and Findeis, 2000), as well as the administration of $A\beta$ antibodies to disassemble fibrillar $A\beta$, maintain $A\beta$ solubility and to block the toxic effects of $A\beta$ (Frenkel et al., 1999). However, recently a Phase II clinical trial using a vaccination approach where $A\beta1-42$ was injected into individuals in the early stages of Alzheimer's disease was terminated because of cerebral inflammation observed in some patients.

Thus, despite these advances in the art, to date, there is no cure or effective therapy for reducing a patient's amyloid burden or preventing amyloid deposition in AD. Moreover, even the unequivocal diagnosis of AD can only be made after postmortem examination of brain tissues for the hallmark neurofibrillary tangles (NFT) and neuritic plaques.

Thus, there exists a need in the art for developing effective methods for reducing a patient's amyloid burden.

3. SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that amyloid diseases can be treated by removing A β peptides from a patient's bloodstream. This can be accomplished by 5 the administration of compounds that are associated with A β in order to bind to free A β in circulation. This binding of circulating A β results in the removal of A β from the patient's bloodstream. Free A β can also be removed from a patient's bloodstream by dialysis. Both methods lead to an efflux of A β from the affected organs, resulting in the reduction of a patient's amyloid burden.

10 In one aspect the present invention provides a method for treating a patient suffering from an amyloid disease comprising administering to a patient in need of such treatment a therapeutically effective amount of a compound or fragment thereof associated with A β .

15 In another aspect, the present invention provides a method for treating a patient suffering from an amyloid disease comprising filtering the blood of said patient through a filter, membrane or column thereby removing A β from the patient's blood.

These and other aspects of the present invention will be apparent to those of ordinary skill in the art in the following description, claims and drawings.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1(A-F) are a series of binding curves for the interactions of apoE3 and 20 apoE4 with immobilized A β peptides.

5. **DETAILED DESCRIPTION OF THE INVENTION**

Definitions

The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional 5 guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them.

The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For 10 example, "about" can mean within 1 or more than 1 standard deviations, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value.

15 "Treatment" is defined herein as administration of peptides associated with amyloid disease or dialysis of blood from individuals suffering from amyloid diseases as defined above or prophylactic treatment to those at risk for the diseases, i.e., familial Alzheimer's disease.

In accordance with the present invention there may be employed conventional 20 molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook *et al.*, 1989"); *DNA Cloning: A practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* [B.D. Hames & S.J. Higgins eds.(1985)]; *Transcription and Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells and Enzymes* [IRL Press, (1986)]; B.

Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

Molecular Biology

A "nucleic acid molecule" refers to the phosphate ester polymeric form of 5 ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in 10 particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found *inter alia*, in linear (*e.g.*, restriction fragments) or circular DNA molecules, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA 15 molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

A polynucleotide" or "nucleotide sequence" is a series of nucleotide bases (also called "nucleotides") in a nucleic acid, such as DNA and RNA, and means any chain of two or 20 more nucleotides. A nucleotide sequence typically carries genetic information, including the information used by cellular machinery to make proteins and enzymes. These terms include double or single stranded genomic and cDNA, RNA, any synthetic and genetically manipulated polynucleotide, and both sense and anti-sense polynucleotide (although only sense strands are being represented herein). This includes single- and double-stranded molecules, *i.e.*, DNA-25 DNA, DNA-RNA and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid backbone. This also includes nucleic acids containing modified bases, for example thio-uracile, thio-guanine and fluoro-uracil.

The nucleic acids herein may be flanked by natural regulatory (expression control) sequences, or may be associated with heterologous sequences, including promoters,

internal ribosome entry sites (IRES) and other ribosome binding site sequences, enhancers, response elements, suppressors, signal sequences, polyadenylation sequences, introns, 5'- and 3'- non-coding regions, and the like. The nucleic acids may also be modified by many means known in the art. Non-limiting examples of such modifications include methylation, "caps",
5 substitution of one or more of the naturally occurring nucleotides with an analog, and internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoroamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.). Polynucleotides may contain one or more additional covalently linked moieties, such as, for example, proteins (e.g., nucleases, toxins,
10 antibodies, signal peptides, poly-L-lysine, etc.), intercalators (e.g., acridine, psoralen, etc.), chelators (e.g., metals, radioactive metals, iron, oxidative metals, etc.), and alkylators. The polynucleotides may be derivitized by formation of a methyl or ethyl phosphotriester or an alkyl phosphoramidate linkage. Furthermore, the polynucleotides herein may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels
15 include radioisotopes, fluorescent molecules, biotin, and the like.

A "promoter" or "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction)
20 to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. The promoter may be operatively associated with other expression control sequences, including
25 enhancer and repressor sequences.

Promoters which may be used to control gene expression include, but are not limited to, cytomegalovirus (CMV) promoter (U.S. Patents No. 5,385,839 and No. 5,168,062), the SV40 early promoter region (Benoist and Chambon, Nature 1981, 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., Cell 30 1980, 22:787-797), the herpes thymidine kinase promoter (Wagner et al., Proc. Natl. Acad. Sci.

USA 1981, 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, Nature 1982, 296:39-42); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Komaroff, *et al.*, Proc. Natl. Acad. Sci. USA 1978, 75:3727-3731), or the *tac* promoter (DeBoer, *et al.*, Proc. Natl. Acad. Sci. USA 1983, 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and transcriptional control regions that exhibit hematopoietic tissue specificity, in particular: beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, Nature 1985, 315:338-340; Kollias *et al.*, Cell 1986, 46:89-94), hematopoietic stem cell differentiation factor promoters, erythropoietin receptor promoter (Maouche *et al.*, Blood 1991, 15:2557), etc.

A "coding sequence" or a sequence "encoding" an expression product, such as a RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, results in the production of that RNA, polypeptide, protein, or enzyme, i.e., the nucleotide sequence encodes an amino acid sequence for that polypeptide, protein or enzyme. A coding sequence for a protein may include a start codon (usually ATG) and a stop codon.

The term "gene", also called a "structural gene" means a DNA sequence that codes for or corresponds to a particular sequence of amino acids which comprise all or part of one or more proteins or enzymes, and may or may not include regulatory DNA sequences, such as promoter sequences, which determine for example the conditions under which the gene is expressed. Some genes, which are not structural genes, may be transcribed from DNA to RNA, but are not translated into an amino acid sequence. Other genes may function as regulators of structural genes or as regulators of DNA transcription.

A coding sequence is "under the control of" or "operatively associated with" transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into RNA, particularly mRNA, which is then trans-RNA spliced (if it contains introns) and translated into the protein encoded by the coding sequence.

As used herein, the term "isolated" means that the referenced material is removed from its native environment, e.g., a cell. Thus, an isolated biological material can be free of

some or all cellular components, i.e., components of the cells in which the native material is occurs naturally (e.g., cytoplasmic or membrane component). A material shall be deemed isolated if it is present in a cell extract or if it is present in a heterologous cell or cell extract. In the case of nucleic acid molecules, an isolated nucleic acid includes a PCR product, an isolated 5 mRNA, a cDNA, or a restriction fragment. In another embodiment, an isolated nucleic acid is preferably excised from the chromosome in which it may be found, and more preferably is no longer joined or proximal to non-coding regions (but may be joined to its native regulatory regions or portions thereof), or to other genes, located upstream or downstream of the gene contained by the isolated nucleic acid molecule when found in the chromosome. In yet another 10 embodiment, the isolated nucleic acid lacks one or more introns. Isolated nucleic acid molecules include sequences inserted into plasmids, cosmids, artificial chromosomes, and the like, i.e., when it forms part of a chimeric recombinant nucleic acid construct. Thus, in a specific embodiment, a recombinant nucleic acid is an isolated nucleic acid. An isolated protein may be associated with other proteins or nucleic acids, or both, with which it associates in the cell, or 15 with cellular membranes if it is a membrane-associated protein. An isolated organelle, cell, or tissue is removed from the anatomical site in which it is found in an organism. An isolated material may be, but need not be, purified.

The term "purified" as used herein refers to material that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, 20 including native materials from which the material is obtained. For example, a purified protein is preferably substantially free of other proteins or nucleic acids with which it is associated in a cell; a purified nucleic acid molecule is preferably substantially free of proteins or other unrelated nucleic acid molecules with which it can be found within a cell. As used herein, the term "substantially free" is used operationally, in the context of analytical testing of the material. 25 Preferably, purified material substantially free of contaminants is at least 50% pure; more preferably, at least 90% pure, and more preferably still at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, and other methods known in the art.

Methods for purification are well-known in the art. For example, nucleic acids 30 can be purified by precipitation, chromatography (including without limitation preparative solid

phase chromatography, oligonucleotide hybridization, and triple helix chromatography), ultracentrifugation, and other means. Polypeptides and proteins can be purified by various methods including, without limitation, preparative disc-gel electrophoresis and isoelectric focusing; affinity, HPLC, reversed-phase HPLC, gel filtration or size exclusion, ion exchange 5 and partition chromatography; precipitation and salting-out chromatography; extraction; and countercurrent distribution. For some purposes, it is preferable to produce the polypeptide in a recombinant system in which the protein contains an additional sequence tag that facilitates purification, such as, but not limited to, a polyhistidine sequence, or a sequence that specifically binds to an antibody, such as FLAG and GST. The polypeptide can then be purified from a 10 crude lysate of the host cell by chromatography on an appropriate solid-phase matrix.

Alternatively, antibodies produced against the protein or against peptides derived therefrom can be used as purification reagents. Cells can be purified by various techniques, including centrifugation, matrix separation (e.g., nylon wool separation), panning and other immunoselection techniques, depletion (e.g., complement depletion of contaminating cells), and 15 cell sorting (e.g., fluorescence activated cell sorting (FACS)). Other purification methods are possible and contemplated herein. A purified material may contain less than about 50%, preferably less than about 75%, and most preferably less than about 90%, of the cellular components, media, proteins, or other undesirable components or impurities (as context requires), with which it was originally associated. The term "substantially pure" indicates the 20 highest degree of purity which can be achieved using conventional purification techniques known in the art.

The term "express" and "expression" means allowing or causing the information in a gene or DNA sequence to become manifest, for example producing RNA (such as rRNA or mRNA) or a protein by activating the cellular functions involved in transcription and translation 25 of a corresponding gene or DNA sequence. A DNA sequence is expressed by a cell to form an "expression product" such as an RNA (e.g., a mRNA or a rRNA) or a protein. The expression product itself, e.g., the resulting RNA or protein, may also said to be "expressed" by the cell.

The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the 30 vector and introduced to the host cell. Common expression systems include E. coli host cells and

plasmid vectors, insect host cells such as Sf9, Hi5 or S2 cells and Baculovirus vectors, Drosophila cells (Schneider cells) and expression systems, fish cells and expression systems (including, for example, RTH-149 cells from rainbow trout, which are available from the American Type Culture Collection and have been assigned the accession no. CRL-1710) and 5 mammalian host cells and vectors.

The term "transfection" means the introduction of a foreign nucleic acid into a cell. The term "transformation" means the introduction of a "foreign" (i.e., extrinsic or extracellular) gene, DNA or RNA sequence into a host cell so that the host cell will express the introduced gene or sequence to produce a desired substance, in this invention typically an RNA 10 coded by the introduced gene or sequence, but also a protein or an enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences (e.g., start, stop, promoter, signal, secretion or other sequences used by a cell's genetic machinery). The gene or sequence may include nonfunctional sequences or sequences with no known function. A host 15 cell that receives and expresses introduced DNA or RNA has been "transformed" and is a "transformant" or a "clone". The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell or cells of a different genus or species.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by 20 which a DNA or RNA sequence (e.g., a foreign gene) can be introduced into a host cell so as to transform the host and promote expression (e.g., transcription and translation) of the introduced sequence. Vectors may include plasmids, phages, viruses, etc. and are discussed in greater detail below.

Amyloid-Beta (A β) Peptides

25 A β is a 4.1-4.3 kDa hydrophobic peptide that is encoded on chromosome 21 as part of a much longer amyloid precursor protein APP (Muller-Hill et al., 1989). The APP protein starts with a leader sequence (signal peptide), followed by a cysteine-rich region, an acidic-rich domain, a protease inhibitor motif, a putative N-glycosylated region, a transmembrane domain, and finally a small cytoplasmic region. The A β sequence begins close

to the membrane on the extracellular side and ends within the membrane. Two-thirds of A β faces the extracellular space, and the other third is embedded in the membrane (Kang et al., 1987 and Dyrks et al., 1988). Several lines of evidence suggest that amyloid may play a central role in the early pathogenesis of AD.

5 The present invention provides methods of treating an individual suffering from an amyloid disease by removing A β present in the individuals bloodstream. According to one embodiment of the invention, compounds associated with A β (hereinafter alternatively referred to as "binding compounds"), or fragments of such compounds, are administered to a patient suffering from or at risk for an amyloid disease. Compounds or fragments associated with A β
10 peptides include apolipoprotein E, apolipoprotein J, serum amyloid P component, RNA aptamers directed against A β , α 1- antichymotrypsin, proteoglycans, gangliosides, vimentin, vitronectin and combination thereof.

Those of ordinary skill in the art will also appreciate that mimetics of these compounds can also be used. For example, when the compound has a peptide backbone, the
15 peptide bonds can be replaced with non-peptide bonds. Peptidomimetics can have various different structures (Ripka et al., 1998). For example, peptidomimetics can be: (1) peptide analogues containing one or more amide bond replacements (Spatola, 1983); (2) peptide analogues with various conformational restraints (Hart and Rich 1996), (3) novel structures that replace the entire peptide backbone while retaining isosteric topography of the peptide (Farmer,
20 1980), and (4) various heterocyclic natural products or screening leads that mimic the function of the natural peptide (Fletcher and Campbell, 1998).

RNA aptamers directed against A β can be used to treat amyloid disease pursuant to the present invention. RNA aptamers directed against A β are high affinity ligands selected from a combinatorial library described in Ylera et al. (2002). Such RNA aptamers can be
25 isolated as disclosed in Ylera et al. (supra) or can be chemically synthesized since Ylera et al. provide the nucleotide sequence of a number of A β specific RNA aptamers.

The term "fragment" as used herein refers to the compounds of the present invention which are peptides containing less than the full amino acid sequence of the active

parent protein but retain their binding activity to A β . Fragments or their mimetics can be detected by standard ELISA-based binding assays (Tokuda et al., 2002); phage display techniques (Rodi et al., 1999); yeast two hybrid systems (Uetz et al., 2002), and/or protein microarray technology (Templin et al., 2002). These assays can also be used to screen for novel 5 A β binding compounds.

Following administration, the compound will bind to A β not bound to plasma proteins (free A β). Normally, equilibrium is presumed to exist between free A β in circulation and A β within the brain or other affected organs. Without wishing to be bound by theory, it is believed that the reduction in free A β in the circulation will result in an efflux of A β out of the 10 brain or other similarly affected organs to re-establish the equilibrium. The bound A β will be broken down in the liver and excreted. The subsequent reduction in A β within, e.g., the brain leaves less A β available for aggregation/fibril formation.

Treatment pursuant to the present invention is expected to result in reduced amyloid burden within the brain of an Alzheimer's patient and has the potential to halt or slow 15 the progression of the cognitive impairments observed in the disease. In other amyloid diseases, this treatment approach is expected to enhance clearance of the respective amyloid proteins from their target organs in a similar manner and therefore improve the condition of those patients.

Binding Compounds

Non-limiting examples of the A β binding compounds for use in present invention 20 are apolipoprotein E, apolipoprotein J, serum amyloid P component, RNA aptamers directed against A β , α 1- antichymotrypsin, proteoglycans, gangliosides (such as monosialoganglioside GM1), vitronectin, vimentin, and combinations thereof. These are shown in Table 1 below along with commercial sources therefor.

Table 1

Amyloid- β binding compound	Company	Source
Serum Amyloid P	Biogenesis	Human serum
α 1-antichymotrypsin	Biodesign, USBio, Biogenesis, ICN, Cortex, Scipac	Human Plasma

Apolipoprotein E	ICN, USBio, Biodesign, Fitzgerald, Biogenesis, Cortex	Human plasma, human recombinant
Vitronectin	Calbiochem, Chemicon, Promega, Sigma	Human plasma
Apolipoprotein E4	ICN	Human recombinant
Apolipoprotein E3	ICN	Human recombinant
Apolipoprotein E2	Biogenesis	Human recombinant
Apolipoprotein J	Quidel	Human, purified
Heparan Sulfate Proteoglycan	Sigma	Mouse sarcoma
Monosialoganglioside GM1	Sigma	Bovine brain
Monosialoganglioside GM2	Sigma	Bovine brain
Monosialoganglioside GM3	Sigma	Bovine brain
Disialoganglioside GD1a	Sigma	Bovine brain
Disialoganglioside GD1b	Sigma	Bovine brain
Trisialoganglioside GT1b	Sigma	Bovine brain
Gangliosides mixture	Sigma	Bovine brain
Vimentin	Sigma, Biodesign, Biogenesis	Bovine lens

In addition to these sources, these compounds can be purified from human plasma and/or various human tissues.

Moreover, compounds which are proteins can be produced recombinantly using expression systems known to those of ordinary skill in the art disclosed above and the DNA sequences set forth in public databases (<http://www.ncbi.nlm.nih.gov/LocusLink/>)

The recombinant proteins can be purified using standard techniques well known to those of ordinary skill in the art disclosed above:

10 The plasma concentrations of A β binding compounds and soluble A β (sA β) are as follows:

Proteins/Peptides	Mean Plasma Levels (μ g/ml)
Apolipoprotein E (monomer)	54
Apolipoprotein E	54

(dimer)	
Apolipoprotein J	100
$\alpha 1$ -antichymotrypsin	40
serum amyloid P component	34
sA β	.001

The compounds of the present invention may be administered systemically. The term "systemic" as used herein includes parenteral, topical, oral, spray inhalation, rectal, nasal and bucal administration. The term "parenteral" as used herein includes subcutaneous,

- 5 intravenous, intramuscular, and intraperitoneal administration. Preferably, the compositions are administered orally or intravenously in effective amounts to treat the amyloid diseases. An effective amount to treat the diseases would broadly range between about 0.1 mg and about 10 mg per kg body weight of the recipient per day and may be administered as a single or divided doses. Specifically, the amount administered would range between about one-tenth and up to
- 10 about two-fold of mean plasma levels of the A β binding compound. The amount administered of any of the compounds would be no greater than needed to bind all free A β in plasma. The treatments should be continued throughout the life of the patient.

Dialysis

- In an alternate, preferred embodiment, a reduction in free A β may be achieved not
- 15 by administering the compounds of the present invention but by dialyzing a patient's blood through a column and/or membrane to remove the A β protein from the patient's blood. The column or membrane may contain the amyloid-binding compounds of the present invention covalently attached thereto. Using this approach, the patient will not be directly exposed to these endogenous compounds. The reduction of free A β as a result of dialysis will result in an efflux of
- 20 A β out of the brain or similarly affected organs in order to re-establish the equilibrium. The subsequent reduction in free A β by virtue of dialyzation leaves less A β available for aggregation/fibril formation.

The dialyzing blood treatments of the invention can be used to reduce or eliminate the presence of specific A_β peptides free flowing in plasma. Dialysis eliminates the concerns over adverse immune response or other adverse responses to synthetic constructs or monoclonal antibodies because such constructs or monoclonal antibodies are not introduced into the patient's body. Furthermore, dialysis allows instant initiation and cessation of treatment. Preferred methods of dialysis which may be used in the present invention include, but are not limited to, hemodialysis, plasma perfusion, and hemofiltration. The latter two technologies do not require A_β binding compounds. The methods may be conducted on a continuous or batch basis. Treated blood may be returned to the patient concurrently with treatment or following treatment. The blood may be supplemented or reconstituted with components from donated blood, artificial or synthetic components.

Hemodialysis is the most common method used to treat advanced and permanent kidney failure. It consists of two compartments separated by a semi-permeable membrane. One compartment is filled with blood, the other is filled with a solution of certain minerals and water (referred to as the dialysate bath). Normal blood is 90% water. Water molecules will pass through the membrane freely back and forth. Blood also contains white and red blood cells, protein, fat, sugar, minerals and waste products. The red and white blood cells are too large to pass through the membrane so they remain in the blood compartment. The same is true of fat and protein molecules. However, electrolytes, because of their smaller size, pass freely through the membrane in both directions (principle of diffusion). This principle states that particles in a solution of high concentration pass through a semi-permeable membrane into a solution of lower concentration until there is an equal concentration of particles on both sides (concentration gradient). The concentration of electrolytes is adjusted in the bath side to approximate the levels in normal human blood serum. Metabolic waste products (urea, creatinine etc..) in the blood (larger molecules but small enough to pass through the membrane) are removed utilizing the principle of diffusion. When the concentration of the waste products reach the levels of the blood, the bath solution is changed either periodically or continuously.

For use in the present invention, A_β binding compound are added to the dialysis bath. The semi-permeable membrane will have a molecular weight cutoff at 10,000 Daltons.

Soluble free A β monomers and dimers in the blood will diffuse into the dialysis bath and bind to the A β binding compounds. Thereafter, A β will not diffuse back into the blood.

In hemofiltration procedures, the principle used to eliminate the waste products is different. Solute (in most cases the blood) is carried across a semi-permeable membrane in 5 response to a transmembrane pressure gradient (a process known as solvent drag). This mimics what actually happens in the normal human kidney. The rate of the ultrafiltration depends upon blood flow. This is very effective in removal of fluid and middle sized molecules, which are thought to cause uremia.

When this method is used, there is no need for A β binding compounds. The 10 membranes that are normally used in this technique allow the passage of molecules with a molecular weight of less than 20,000 Daltons. Filtration across the membrane is convective, which means that it is unidirectional. Therefore, filtered A β cannot flow back.

Plasma Perfusion (Plasma exchange or plasmapheresis): Plasma is the fluid portion of the blood that allows circulation of red blood cells, white blood cells and platelets. It 15 consists of mainly water and numerous chemical compounds. Plasma exchange involves the separation and removal of the plasma from the blood in order to remove disease substances circulating in the plasma. The red and white blood cells and platelets are returned to the patient, along with a replacement fluid. Plasma exchange is accomplished with a device called a blood cell separator. Centrifuge or membrane filters are used to separate plasma from cellular blood 20 components. Blood is drawn from a patient's arm vein by a needle which is attached to a blood tubing set. After it goes through the blood cell separator, the cellular components are drawn from the compartment and replacement fluid prescribed by the physician is added to the cellular components. The mix is returned to the patient usually through a needle. All the steps mentioned above can be done in an automated, continuous and safe manner.

25 When plasma perfusion is used pursuant to the present invention, A β binding compounds are not needed. This approach involves removing the plasma from the patient, while the blood cells and platelets are returned to the patient with replacement fluid.

A typical protocol for dialysis would comprise selecting patients who have Down's syndrome, mild cognitive impairment or those at risk for Alzheimer's disease and conducting dialysis using as a binding member the compounds or fragments thereof associated with A β as defined above. Preferably, the dialysis takes place over a period of 2-3 hours, and is 5 repeated as necessary. Typically, dialysis is conducted every 1-7 days for as long as the concentration of free A β in the patient's blood remains high, e.g., above 0.1-0.5 ng/ml (10-50% of mean plasma level).

The efficacy of the treatment can be evaluated by either evaluating the symptoms of the patient or by measuring the concentration or amount of target molecules (A β) in the 10 patient's blood. The amount of A β in the patient's blood can be determined by enzyme linked immunosorbent assay (ELISA) as described below.

In another preferred embodiment of the present invention, the A β binding compounds are immobilized, i.e., fixed so that neither the binding compounds nor the binding pair travel with the blood. Preferably, the binding partner construct is immobilized on a solid 15 support using covalent or affinity binding. Covalent linkages can be achieved using cyanogen bromide (CNBr) or other activation techniques well known to those of ordinary skill in the art (as disclosed in WO 00/74824, European Patent No. 272 792; U.S. Patent No. 5,122,112), and high affinity interactions such as that between avidin and biotin can be used (as disclosed in WO 00/74824; U.S. Patent No. 6,251,394).

20 To regenerate the solid support containing the binding compounds after use, bound A β may be removed preferably by altering the pH and/or by the use of chaotropic agents. Since the binding compound is not administered to the patient in this embodiment of the present invention, the binding compound may be an antibody. Such antibodies are commercially available from numerous sources such as Bachem, Biogenesis, Biosource, Calbiochem, 25 Chemicon and Sigma. When the binding partner is an antibody, it may be attached via its Fc region to a solid support or membrane.

The solid support utilized in dialysis devices and methods can be made out of a variety of substances (nitrocellulose, cellulose, nylon, plastic, rubber, polyacrylamide, agarose, poly(vinylalcohol-co-ethylene), and can be formed in a variety of shapes, including flat

dialyzers, semi-permeable membranes, semi-permeable hollow fibers, coils, permeable spheres, dialysis membranes, and plasmapheresis filters, optionally using linker molecules such as PEG (polyethelene glycol) to attach the ligand (as disclosed in WO 00/74824). In a hemofiltration device, the solid supports may be, for example, beads, plates, hollow filters, or any combination thereof. One particular method which can be used in the present invention is designed to remove small, non-protein-bound toxins using hollow-fiber technology as disclosed in U.S. Patent No. 5,919,369.

The binding compounds described herein can be used in amounts sufficient to remove the targeted molecule ($A\beta$) completely from the blood or simply to reduce the amount of the molecule in the blood. The precise amount of the constructs to be employed depends on the efficiency of the apparatus used and the expected amounts of target molecules in the blood. The amount of binding partner to be immobilized on the solid support can also vary depending on the affinity between binding partner and target, type of perfusion device, and length of perfusion treatment. These amounts can be determined according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. Typically, however, the amount of immobilized binding partner would range between about 50- and about 1000-fold molar excess compared to free $A\beta$ in the blood.

Various designs for hemodialysis and dialysis devices are known and have been described in, e.g., patent literature (see, e.g., U.S. Patent Nos. 4,824,432; 5,122,112; 5,919,369; and 6,287,516; and PCT applications published as WO 90/15631; WO 00/74824; WO 01/37900; and WO 01/45769).

In addition, patients suffering from amyloid disease can be treated by a combination of methods, i.e., administration of the compounds associated with $A\beta$ of the present invention and dialysis. For example, patients can initially be treated using dialysis to rapidly remove the circulating, free $A\beta$ until the amount is stabilized at about 10-50% of the normal value. Thereafter, the compounds of the present invention can be administered, thereby minimizing the number of invasive dialysis treatments.

The present invention will be better understood by reference to the following examples, which are provided as exemplary of the invention, and not to limit the scope thereof.

6. EXAMPLES**EXAMPLE 1**

In the present Example the following materials and methods were used.

Synthetic Peptides and Proteins

5 Peptides DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
(A β 40) and DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (A β 42), identical to residues 672-711 and 672-713 of A β -precursor protein 770 respectively, were synthesized at the W.M. Keck Facility at Yale University (New Haven, CT, U.S.A.) using N-t-butyloxycarbonyl chemistry and purified by HPLC. Aliquots of the final products were
10 lyophilized and stored at -20 °C until use. For preparation of aggregated peptides, 50 μ g of either A β 40 or A β 42 were dissolved in 100 μ l of PBS (0.02 M phosphate buffer, pH 7.4, containing 0.15 M NaCl) and incubated at 37 °C for 72 h. apoE3 and apoE4 produced in SF9
insect cells by the baculovirus expression system were purchased from PanVera (Madison, WI, U.S.A.). In all cases, protein purity was corroborated by SDS/PAGE and N-terminal sequence
15 analysis.

apoE Expressed by Eukaryotic Cell Lines

Human apoE3 or apoE4 were expressed individually in RAW 264 mouse macrophage cells (A.T.C.C. TIB71) stably transfected with genomic DNA encoding the human apoE isoforms, and in human embryonic kidney (HEK) 293 cells (A.T.C.C. CRL1573) stably
20 transfected with cDNA encoding the human apoE isoforms, and harvested in serum-free conditioned medium for each cell line as described (LaDu et al., 1994; LaDu et al., 1995; Smith et al., 1988; Myata et al., 1996). Concentrations of secreted apoE were determined by capture-ELISA (Apotek ApoE; PerImmune, Rockville, MD, U.S.A.) after incubating the harvested conditioned media with 0.05% β -octyl glucopyranoside for 1 h. Aliquots of conditioned media
25 containing apoE3 or apoE4 were stored at 4 °C and used within 2 weeks of harvesting.

Purification of apoE from RAW-264-Cell Conditioned Media

Polyclonal AB947 anti-apoE antibody (1 ml; Chemicon, Temecula, CA, U.S.A.) was coupled to 2 ml of CNBr-activated Sepharose 4B according to the manufacturer's instructions. Conditioned media containing apoE3 or apoE4 was loaded on to the AB947-affinity matrix. Bound apoE was eluted with 0.2M acetic acid, pH 2.2, and immediately neutralized. The elution profile was monitored at 280 nm and the pertinent fractions were pooled and dialysed against 0.02 M Tris/HCl, pH 8.5, containing 0.1 M NaCl.

Isolation of apoE-Containing Particles from HEK-293-Cell Conditioned Media

Aliquots of serum-free conditioned media from HEK-293 cells stably transfected with human apoE3 or apoE4 cDNA, in which the apolipoproteins constituted approximately 50% of the total protein content, were concentrated 50-fold with Centricon-10 (Amicon; Millipore, Bedford, MA, U.S.A.) as described previously. Particles that contained apoE3 or apoE4 were isolated from the corresponding concentrated conditioned media by FPLC using tandem Superose 6 HR10/30 columns (Pharmacia, Piscataway, NJ, U.S.A.) equilibrated in 0.02 M sodium phosphate, pH 7.4, containing 0.05 M NaCl, 0.03% EDTA and 0.02% sodium azide.

15 Delipidation of apoE Isoforms Purified from Conditioned Media of Eukaryotic Cell Lines and from Baculovirus-Transfected Sf9 Cells

apoE isoforms from RAW-264 and HEK-293 cells, purified as described above, as well as apoE produced in Sf9 insect cells by the baculovirus expression system (PanVera), were delipidated in aqueous state using diethyl ether and ethanol. Briefly, the lipoprotein-containing samples were extracted with an equal volume of a 3:2 (v/v) diethyl ether/ethanol mixture, followed by four subsequent extractions of the aqueous phase with a 3:1 (v/v) diethyl ether/ethanol solution. After the final extraction, the remaining solvent was evaporated under a N₂ stream and the apoE concentration determined as described above.

Incorporation of apoE Into Reconstituted High-Density Lipoprotein (rHDL) Particles

25 Extraction of total lipids from the human HDL fraction

Human HDL fractions were isolated by preparative gradient ultracentrifugation of plasma obtained from normal healthy subjects, ages 25-40, after a 10-12 h. fast. The HDL fractions were dialysed extensively at 4 °C against PBS containing 1 mM EDTA, and the total lipid fractions (HDL-lipid) were extracted with a mixture of chloroform and methanol (1:2, v/v)

and centrifuged at 1700 g for 5 min. The bottom layer that contained the extracted lipids was collected, dried under a N₂ atmosphere, dissolved in chloroform and stored at -70 °C until use. The amount of total cholesterol, total triacylglycerols and phospholipids in the HDL and HDL-lipid fractions were determined enzymically with Sigma (St. Louis, MO, U.S.A.) diagnostic kits.

5

Preparation of reconstituted apoE-containing HDL particles

rHDL particles containing apoE were prepared as described using recombinant apoE expressed in baculovirus-infected Sf9 cells and the HDL-lipids extracted from human plasma HDL lipoparticles. In a typical experiment, the HDL-lipids (500 µg) were placed in a glass tube, dried under N₂ atmosphere, resuspended in 0.01 M Tris/HCl buffer, pH 8, containing 10 0.15 M NaCl (TBS) and 0.001 M EDTA. After the addition of 280 µg of sodium cholate, the suspension was incubated at 4 °C for 12 h. Subsequently, 500 µg of either Sf9 apoE3 or apoE4 was added to the reaction, incubated at 4 °C for another 12 h, and dialysed extensively at 4 °C against PBS containing 0.01% EDTA. The fractions containing apoE incorporated into 15 lipoparticles (apoE-rHDL) were separated from lipid-free apoE by gel-filtration chromatography using a SUPEROSE 12® column (Pharmacia) equilibrated in 0.02 M phosphate buffer, pH 7.4, containing 0.05M NaCl, 0.03% EDTA and 0.02% sodium azide, at a flow rate of 0.8 ml/min. Collected fractions were analysed by native PAGE using 4-20% Tris/glycine gels and Western-blot analysis employing a monoclonal anti-apoE antibody (3D12; BioDesign, Kennebunk, ME, U.S.A.). The fractions containing apoE-rHDL were pooled for solid-phase binding studies and 20 the concentration of apoE in the lipoparticles was determined using the ApoTek ApoE system as described above.

Chemical cross-linking of rHDL particles

apoE molecules reconstituted into HDL particles were cross-linked using bis(sulphosuccinimidyl) suberate (BS3). Briefly, BS3 was added to the apoE-rHDL fraction at a 25 concentration of 0.002 M in PBS, incubated at room temperature for 4 h., and the reaction stopped by the addition of 0.03 M Tris/HCl buffer, Ph 7.4. After desalting with Microcon 10 (Amicon, Millipore) and lyophilization, the cross-linked samples were separated by Tris/Tricine PAGE (10% polyacrylamide), transferred on to an Immobilon-P membrane (Millipore) and

reacted with monoclonal 3D12 anti-apoE antibody followed by horseradish-peroxidase-conjugated anti-mouse IgG. The Western blot was developed by chemiluminescence using the Super-Signal kit (Pierce, Rockford, IL, U.S.A.).

Solid-Phase Binding Assays

5 The binding of apoE to A β species was studied by ELISA using immobilized freshly prepared (non-aggregated) or 72-h-aggregated A β 40 and A β 42 and apoE3 or apoE4 isoforms with different degrees of lipidation. Polystyrene microtitre plates (Immulon2; Dynex Technology, Chantilly, VA, U.S.A.) were coated for 2 h. at 37 °C with either fresh or aggregated A β 40 and A β 42 (400 ng in 100 μ l of 0.1 M NaHCO₃, pH 9.6, per well). Under these conditions,
10 10 ng of fresh A β 40, 9.6 ng of fresh A β 42, 10.2 ng of aggregated A β 40 and 10.9 ng of aggregated A β 42 (representing 2.5, 2.4, 2.6 and 2.7% of the peptide offered, respectively) were coated to the microtitre wells, as determined by a modification of the Quantigold assay (Diversified Biotech, Boston, MA, U.S.A.) for protein quantification. After blocking with Superblock (Pierce), increasing concentrations of apoE (0-150 nM in TBS; 100 μ l per well) were
15 added to the A β -coated wells and incubated for 3 h. at 37 °C. Bound apoE was detected with monoclonal anti-apoE antibody (3D12, 1:1000) followed by alkaline-phosphatase-conjugated goat F(ab')2 anti-mouse IgG (1:3000; BioSource International, Camarillo, CA, U.S.A.). The reaction was developed for 30 min. with p-nitrophenyl phosphate in diethanolamine buffer (Bio-Rad, Hercules, CA, U.S.A.), and quantified at 405 nm on a Microplate Reader (Cambridge
20 Technology, Watertown, MA, U.S.A.). For Scatchard analysis, bound apoE values were expressed in fmol with the aid of a calibration curve in which known concentrations of apoE coated to microtitre wells (as determined by Quantigold assay) were reacted with 3D12, followed by alkaline-phosphatase-conjugated F(ab')2 anti-mouse IgG under conditions identical with those described above. Under the experimental conditions employed, an excess of apoE was
25 reacted with solid-phase A β ; therefore, only a small fraction of added ligand bound to the immobilized peptide and the concentration of free ligand was considered equivalent to the total apoE added.

RESULTS

Increasing concentrations (0-150nM) of various apoE3 preparations were reacted with microtiter ELISA wells coated with non-aggregated A β 40 or A β 42 for 3 hours. Bound apoE was detected in all cases, with monoclonal 3D12 anti-apoE antibody followed by alkaline phosphatase-labeled anti-mouse IgG. The results are shown in Figure 1.

5 In Figure 1, (A, D) Binding to A β 40 and A β 42, respectively, of Sf9-derived apoE3 and apoE4, both delipidated and upon incorporation into r-HDLs.¹ (B, E) Binding to A β 40 and A β 42, respectively, of HEK-derived apoE3 and apoE4 both, in their native HDL particles and following delipidation. (C, F) Binding to A β 40 and A β 42, respectively, of RAW-derived apoE3 and apoE4, in the native HDL-particles and following delipidation. Each point
10 represents the mean (\pm standard deviation) of triplicates.

These results show the high affinity binding of apoE to A β . Therefore, apoE, and the other compounds disclosed herein, may be used as A β binding compounds for the therapeutic purposes disclosed herein.

When apoE is made recombinantly, it is delipidated. This form of apoE is available commercially. Under physiological conditions or upon incorporation into r-HDL particles it becomes lipitated. ApoE in both forms binds A β with high affinity.

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* * *

5 The present invention is not to be limited in scope by the specific embodiments
described herein. Indeed, various modifications of the invention in addition to those described
herein will become apparent to those skilled in the art from the foregoing description and the
accompanying figures. Such modifications are intended to fall within the scope of the appended
claims. It is further to be understood that all values are approximate, and are provided for
10 description.

Patents, patent applications, publications, product descriptions, and protocols are
cited throughout this application, the disclosures of which are incorporated herein by reference in
their entireties for all purposes.

What is claimed is:

1 1. A method for treating a patient suffering from an amyloid disease comprising
2 administering to a patient in need of such treatment a therapeutically effective amount of a
3 compound associated with amyloid-beta or fragments thereof which binds to free amyloid-beta
4 in said patient's blood and is excreted.

1 2. The method of claim 1 wherein said compound bound to amyloid-beta is
2 excreted.

1 3. The method according to claim 1 wherein the amyloid disease is Alzheimer's
2 disease.

1 4. The method according to claim 1 wherein the compound or fragment thereof
2 associated with amyloid-beta is administered systemically.

1 5. The method according to claim 4 wherein between about 1 mg and about 100 mg
2 per kg body weight of the recipient of the compound or fragment thereof associated with
3 amyloid-beta is administered daily.

1 6. The method according to claim 1 wherein the compound or fragments thereof
2 associated with A β is selected from apolipoprotein E, apolipoprotein J, serum amyloid P
3 component, RNA aptamers directed against A β , α 1- antichymotrypsin, a proteoglycan, a
4 ganglioside, vimentin, vitronectin and combinations thereof.

1 7. The method according to claim 6 wherein the apolipoprotein E is selected from
2 apolipoprotein E2, apolipoprotein E3 or apolipoprotein E4.

1 8. The method according to claim 6 wherein said compound or fragments thereof is
2 a mimetic of said compound or fragments thereof.

1 9. The method according to claim 6 wherein the proteoglycan is a heparan sulfate
2 proteoglycan.

1 10. The method according to claim 6 wherein the ganglioside is selected from
2 monosialoganglioside GM1, monosialoganglioside GM2, monosialoganglioside GM3,

3 disialoganglioside GD1a, disialoganglioside GD1b, trisialoganglioside GT1b, or a ganglioside
4 mixture thereof.

1 11. A method of treating an amyloid disease in a patient in need of such treatment
2 comprising filtering the blood of said patient through a filter, membrane or column, thereby
3 removing amyloid-beta from said patient's.

1 12. The method of claim 11 wherein said filtered blood is returned to said patient.

1 13. The method according to claim 11 wherein the amyloid disease is Alzheimer's
2 disease.

1 14. The method of claim 11 wherein said membrane filter for column is coated with a
2 compound associated with amyloid-beta or a fragment of said compound.

1 15. The method according to claim 14 wherein said compound associated with
2 amyloid-beta is selected from apolipoprotein E, apolipoprotein J, serum amyloid P component, a
3 RNA aptamer directed against A β , α 1- antichymotrypsin, a proteoglycan, a ganglioside,
4 vimentin, vitronectin or combinations thereof.

1 16. The method according to claim 15 wherein the apolipoprotein E is selected from
2 apolipoprotein E2, apolipoprotein E3 and apolipoprotein E4.

1 17. The method according to claim 15 wherein said compound or fragments thereof is
2 a mimetic of said compound or fragments thereof.

1 18. The method according to claim 15 wherein the proteoglycan is a heparan sulfate
2 proteoglycan.

1 19. The method according to claim 15 wherein said ganglioside is selected from
2 monosiologanglioside GM1, monosiologanglioside GM2, monosiologanglioside GM3,
3 disialoganglioside GD1a, disialoganglioside GD1b, trisialoganglioside GT1b, or a ganglioside
4 mixture thereof.

- 1 20. The method according to claim 11 wherein the method of filtering the blood of
2 said patient is selected from hemodialysis, plasma perfusion and hemofiltration.

Abstract

Disclosed herein are methods for treating amyloid disease in humans by clearing amyloid peptides from a patient's bloodstream. In particular, this invention relates to the administration of compounds associated with A β or the dialyzation of blood through a column or membrane in order to remove A β peptides from circulation.

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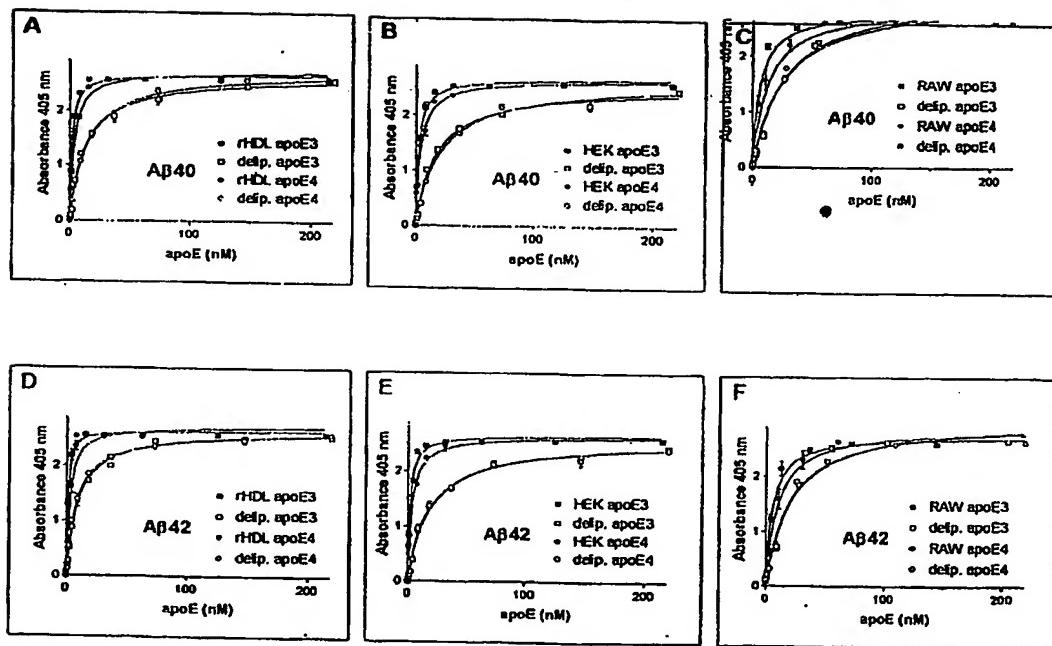


FIG. 1

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